In-situ Detection and Mapping of Microbial Life in Ice with a Miniaturized Biospectrologger

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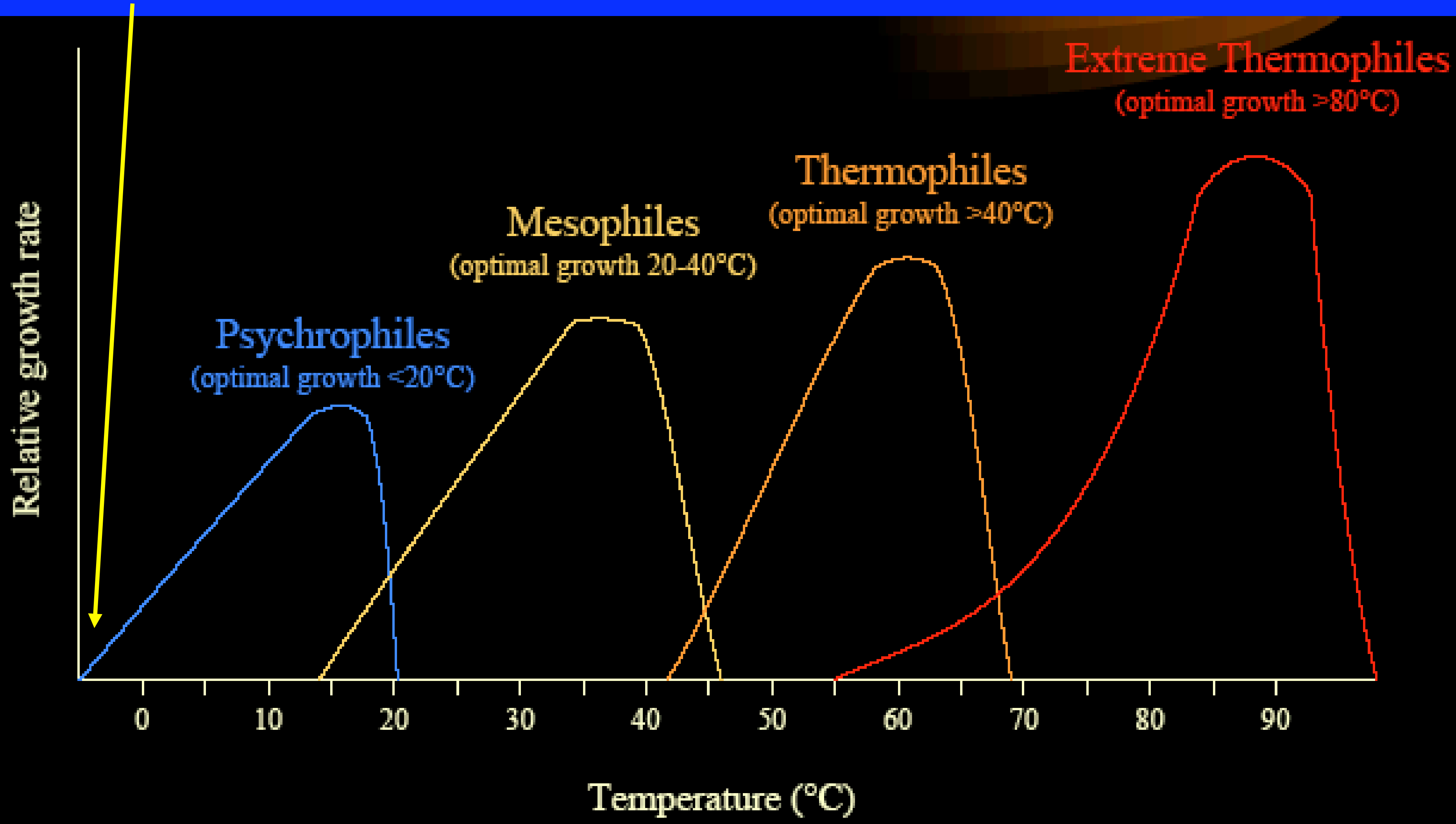
Despite growth curves in pure culture, and despite statements in the literature that the limit for life is ~ -20°C, I will show that it is worthwhile to search for living microbes in cold planets.



Unusual route to invention

↓

AMANDA high-energy neutrino observatory ~1993

↓

Dust in glacial ice governs its optical properties ~1994

↓

Brainstorming for proposed NSF STC “Deep Ice” ~1998

↓ ↓

Microbes in veins in ice ~2000 | Dust logger ~2001

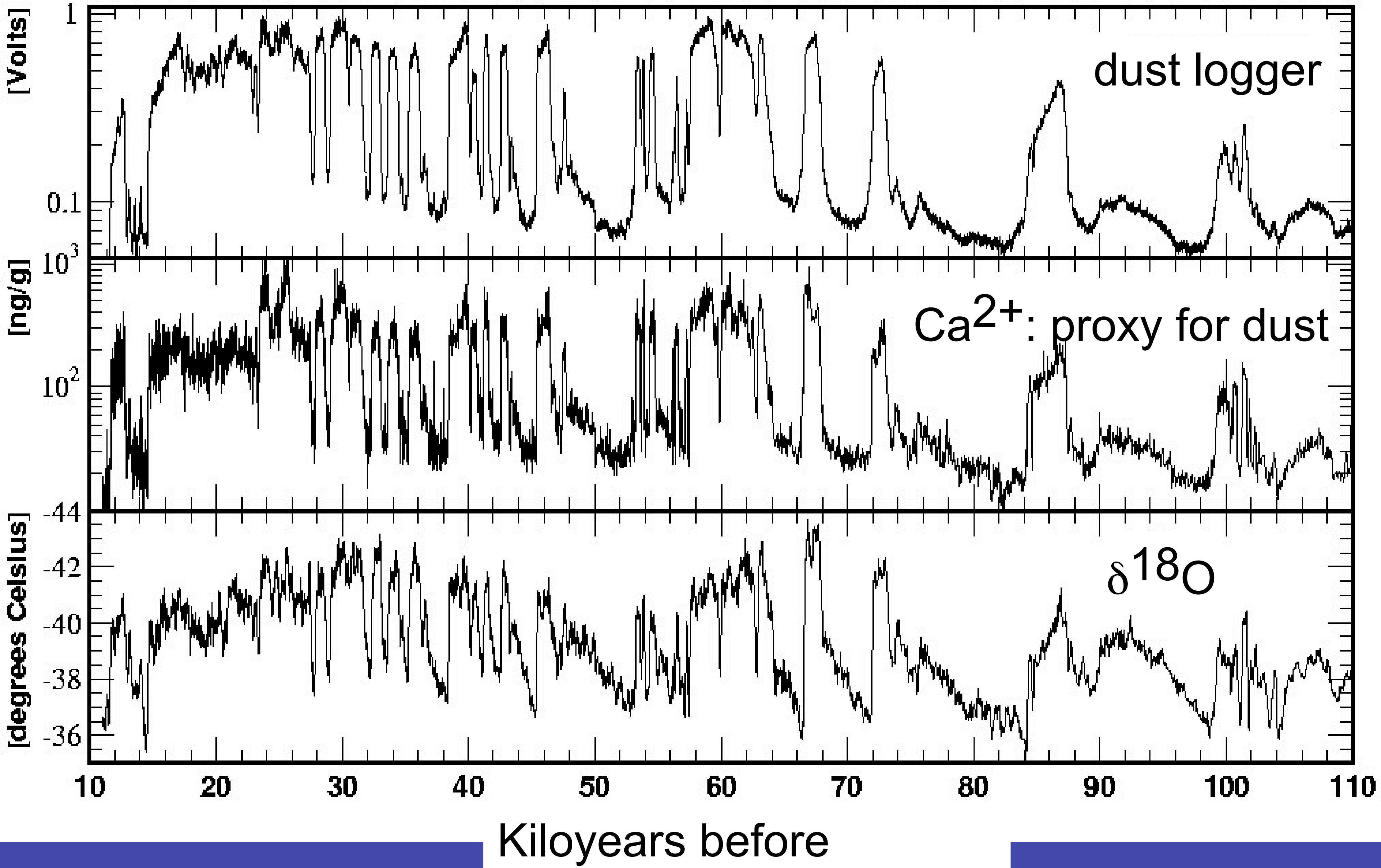
↓ ↓

Biospectral logger (BSL)

↓

Discoveries?

Jumps in dust signal in 3054 m borehole (Greenland) ⇒ abrupt climate changes. Do microbes accompany dust and volcanic ash?



Technology Goal

• Construct biospectral logger (miniBSL) to fit into a 5-cm borehole; 224 nm laser; 6 notch filters + 1 ND filter; 7 PMTs; noninvasive

Science Goals

• Log 300-m-deep borehole in glacial ice 8 km from S. Pole. On Jan. 16, N. Bramall logged this borehole with a large BSL.

• With Peter Doran *et al.*, log 19-m ice cover, microbial mats, and hypersaline water in Lake Vida, Antarctica.

• Scan ice cores at NICL ; do microbes correlate with climate changes, basal ice, volcanic ash, age and T of ice? Do they live in veins in ice?

• From fluorescence spectra, identify types, live/dead ratio, microhabitats, water/ice ratio in subsurface,…

Microbial life on Earth “follows the water”

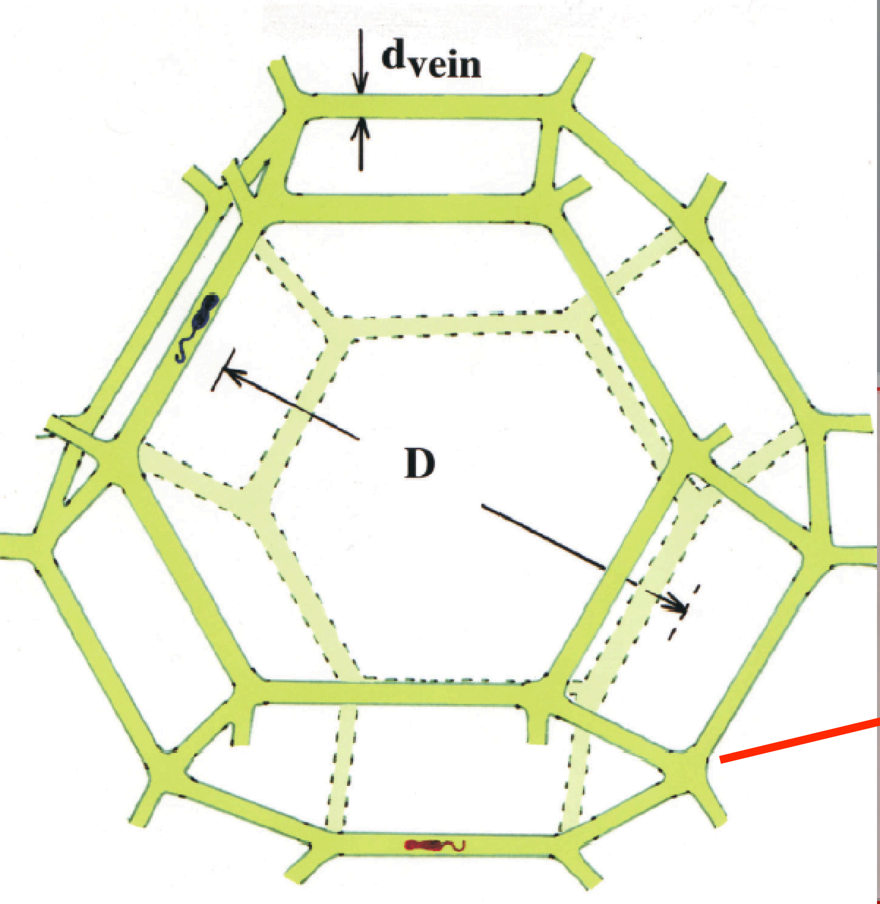
- Ice has microbes in interconnecting aqueous, μm-size veins (salt-rich on Mars; acid-rich on Europa)

- Permafrost has microbes on nm-thick films of unfrozen water at ice/grain surfaces.

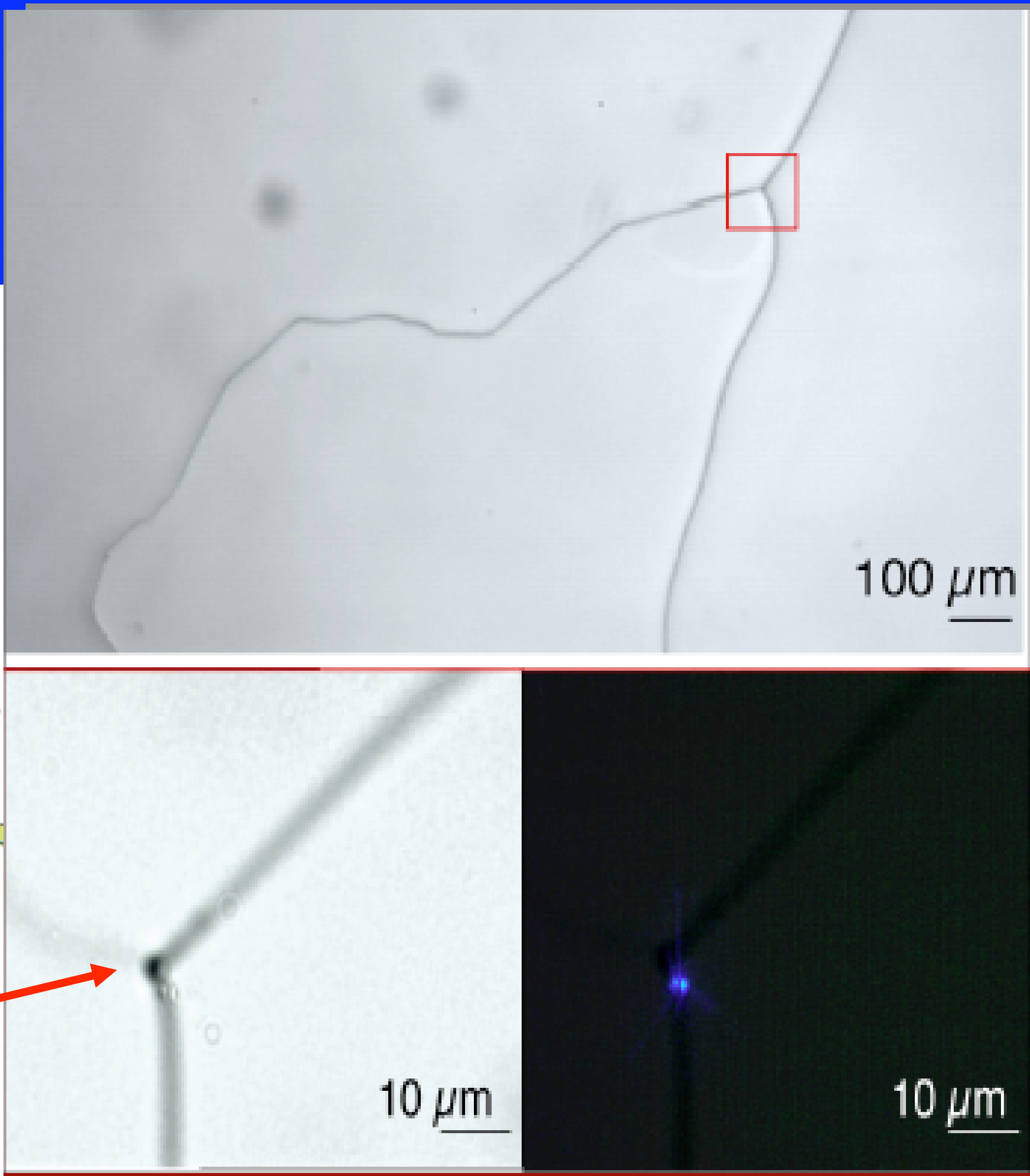
Melting points of some aqueous eutectics

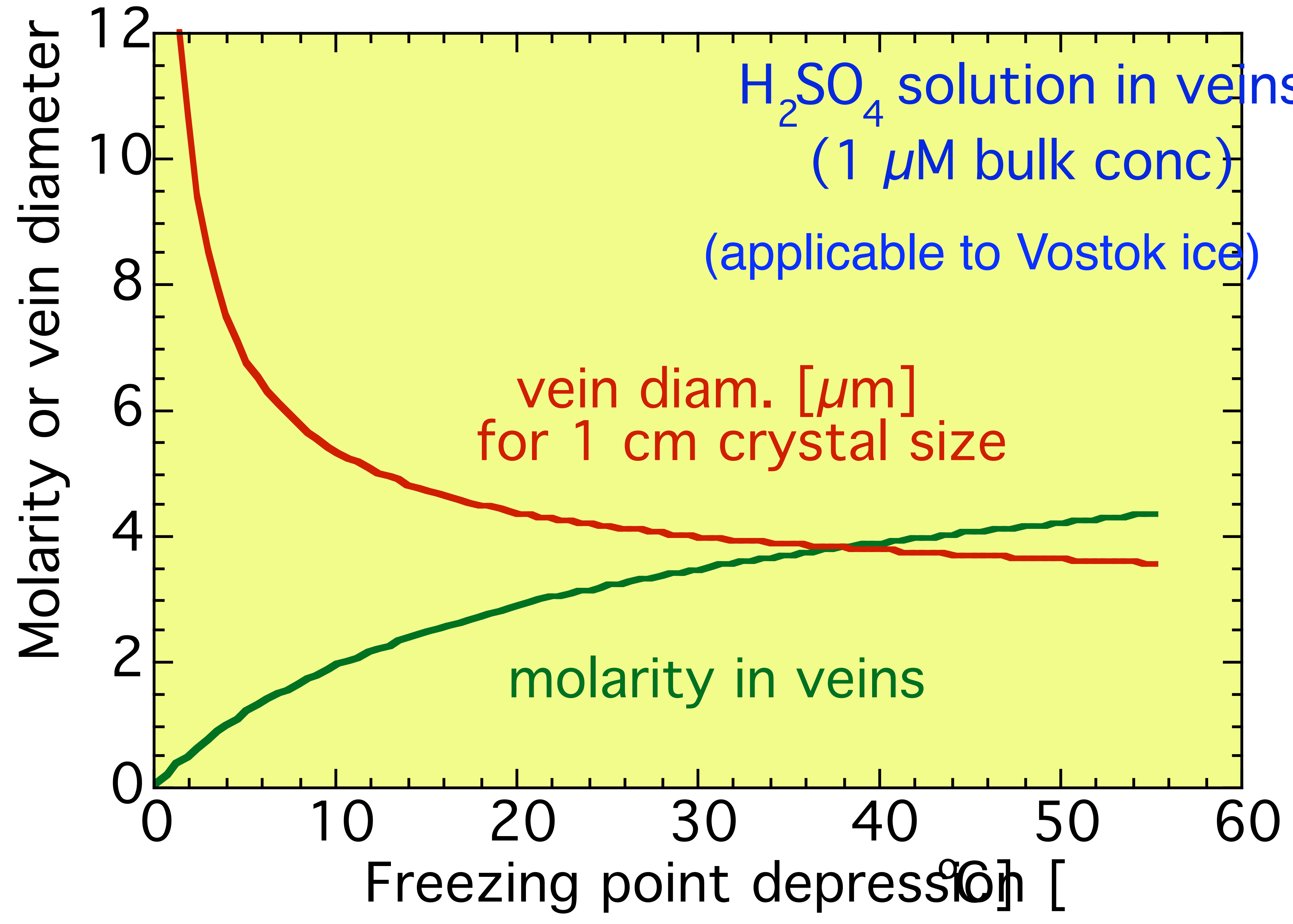
HCl	-90 C
HNO_3	-43 C
H_2SO_4	-73 C
Methanosulfonic acid	-75 C
Formaldehyde	-92 C
Formic acid	-49 C
$NaCl\ 2H_2O$	-22 C
$CaCl_2\ 6H_2O$	-50 C
$MgCl_2 \bullet 12H_2O$	-33 C
$CaCl_2 \bullet 6\ H_2O + MgCl_2 \bullet 12H_2O$	-55 C
NH_4OH	-84 C
NH_4CN	-100 C

Direct evidence that adapted microorganisms can exist and metabolize in a network of liquid veins at T down to -90ºC.

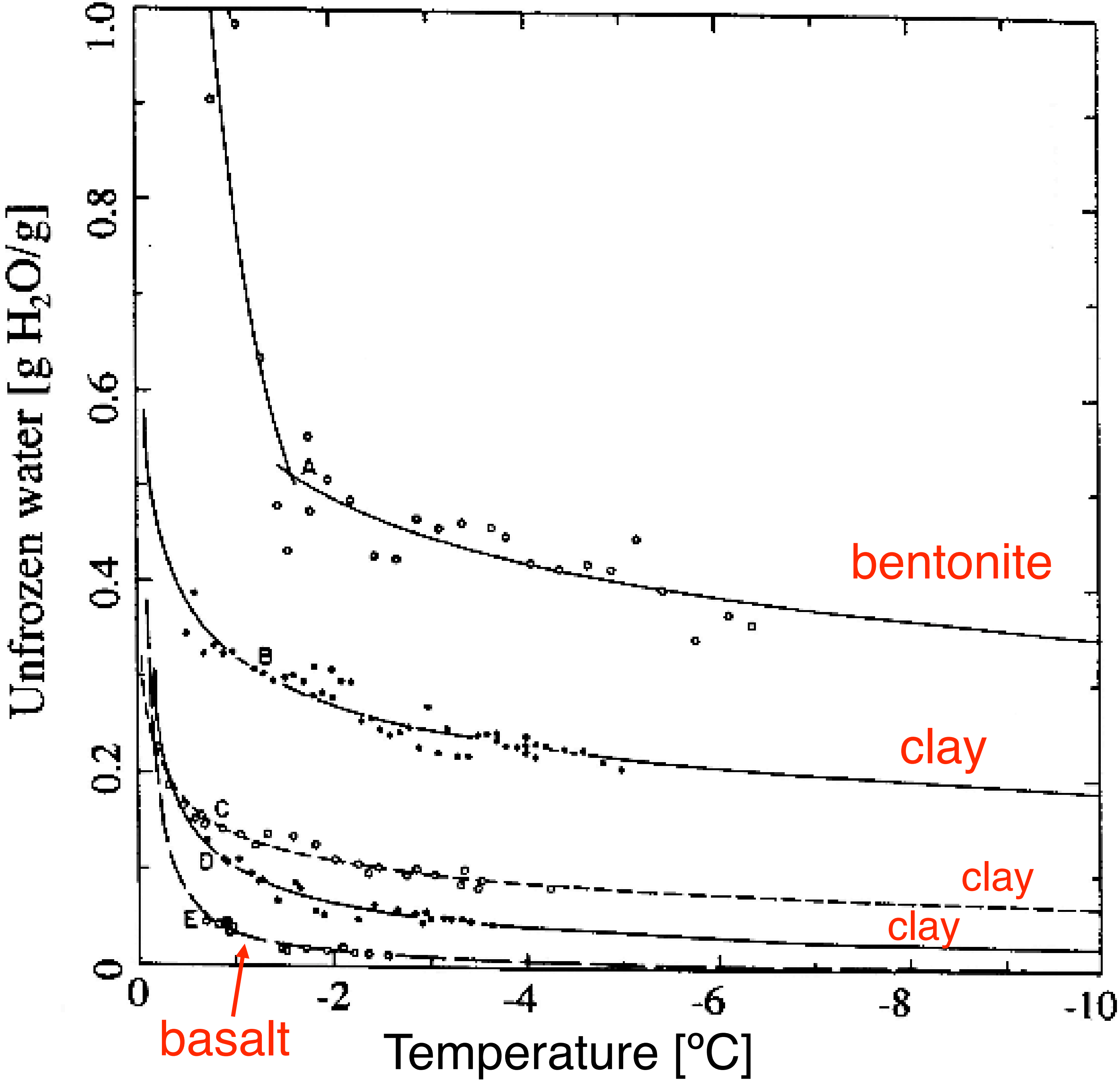


Fluorescing microbe in a vein in sea ice at -15ºC (K. Junge and J. Deming)





H_2SO_4 solution in veins
(1 μM bulk conc)
(applicable to Vostok ice)
vein diam. [μm]
for 1 cm crystal size
molarity in veins
Molarity or vein diameter
Freezing point depression [°C]
0
2
4
6
8
10
12
0
10
20
30
40
50
60



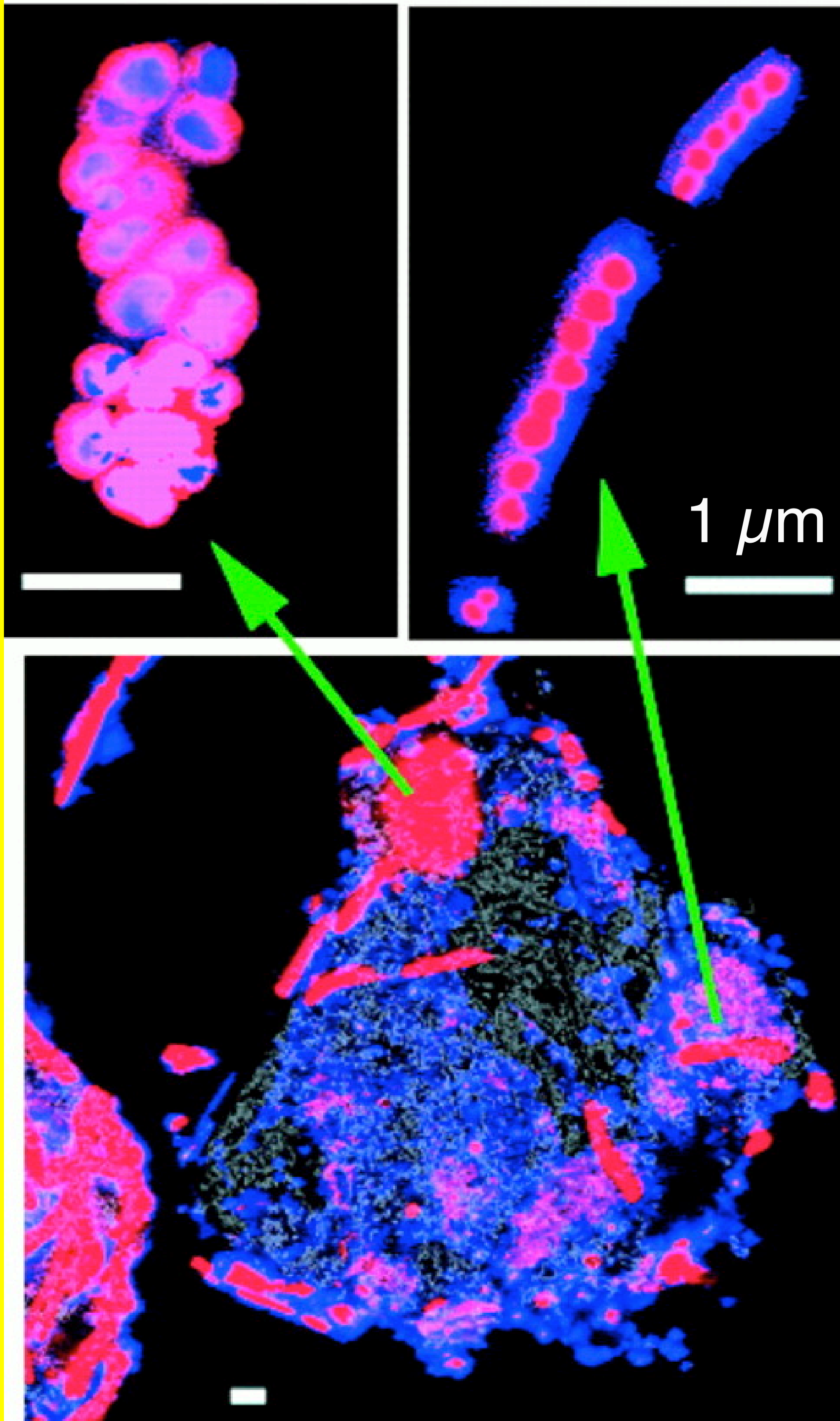
Water film thickness at grain/ice Interface depends on temperature and rock type: clay minerals are best: high surface to volume

Microbes live on mineral surfaces in permafrost and extract energy from the thin films that remain liquid even at low temperature.

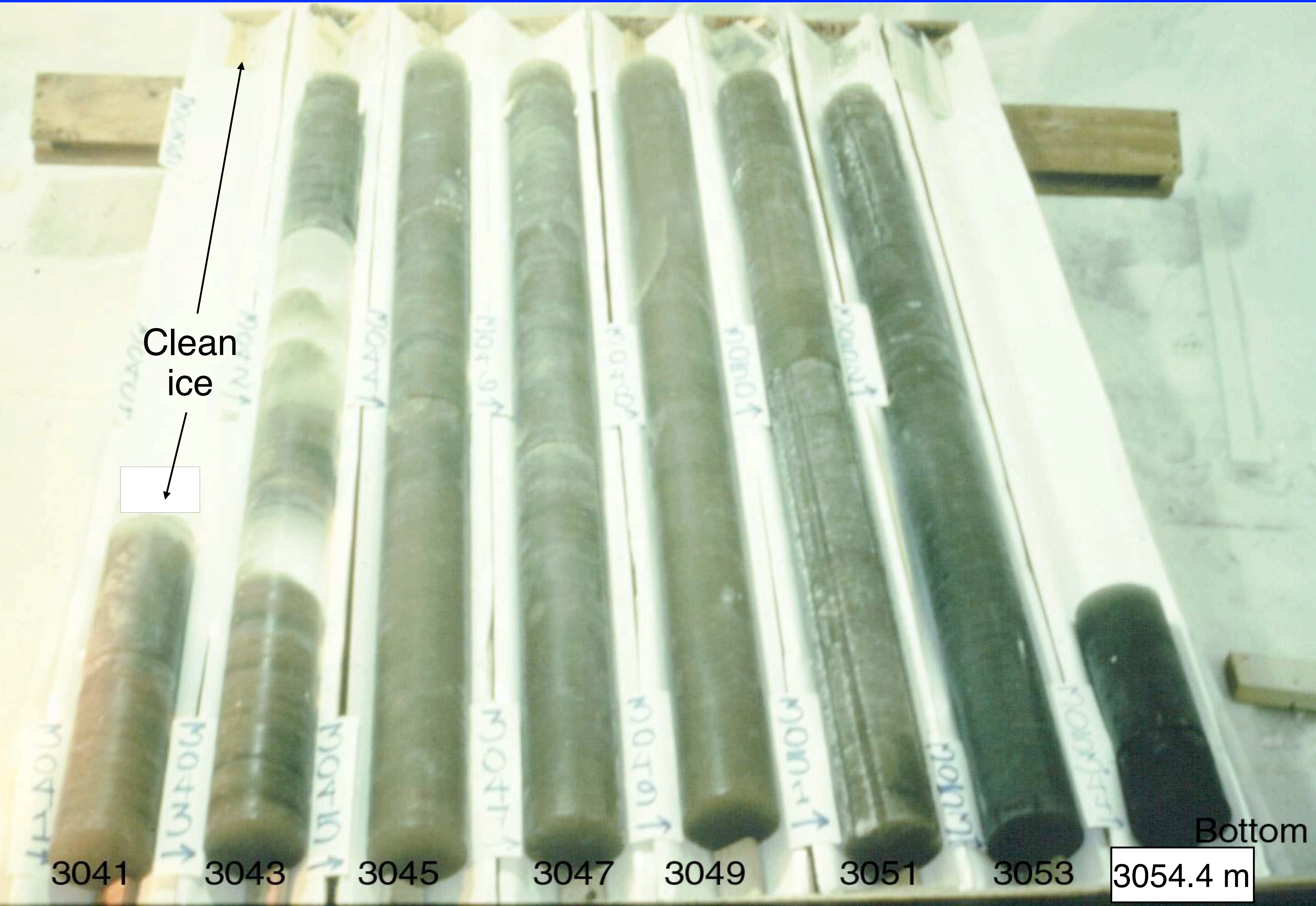
- blue = DAPI-stained bacteria

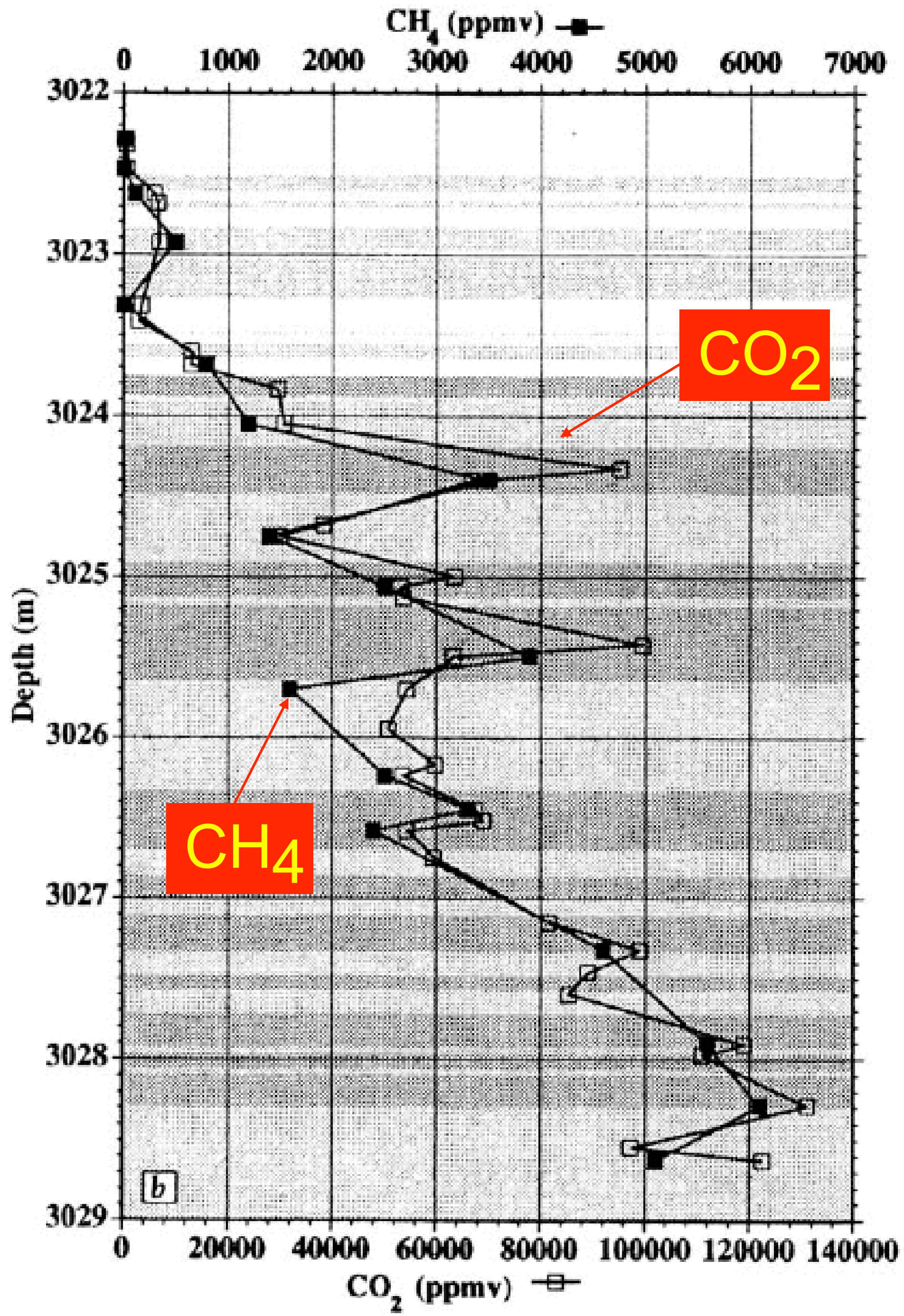
- red = chlorophyll fluorescence

(Priscu et al.: ice-covered Lake Bonney, Antarctica)



GISP2 (Greenland) 3054 m core; silty ice in bottom 13 m





near bottom of Greenland ice core ⇒ microbes are metabolizing in ice.

$C_2H_4O_2+2O_2 \rightarrow 2\mathbf{CO_2}+2H_2O$

$4H_2+CO_2 \rightarrow \mathbf{CH_4}+2H_2O$

V. Miteva finds ~7 x 10^7 cells/cm^3 in deepest ice.

C. Tung (my undergrad) finds, on average, ~20 cells

Our geochemical method for inferring metabolic rate of trapped, dormant microbes at low temperature:

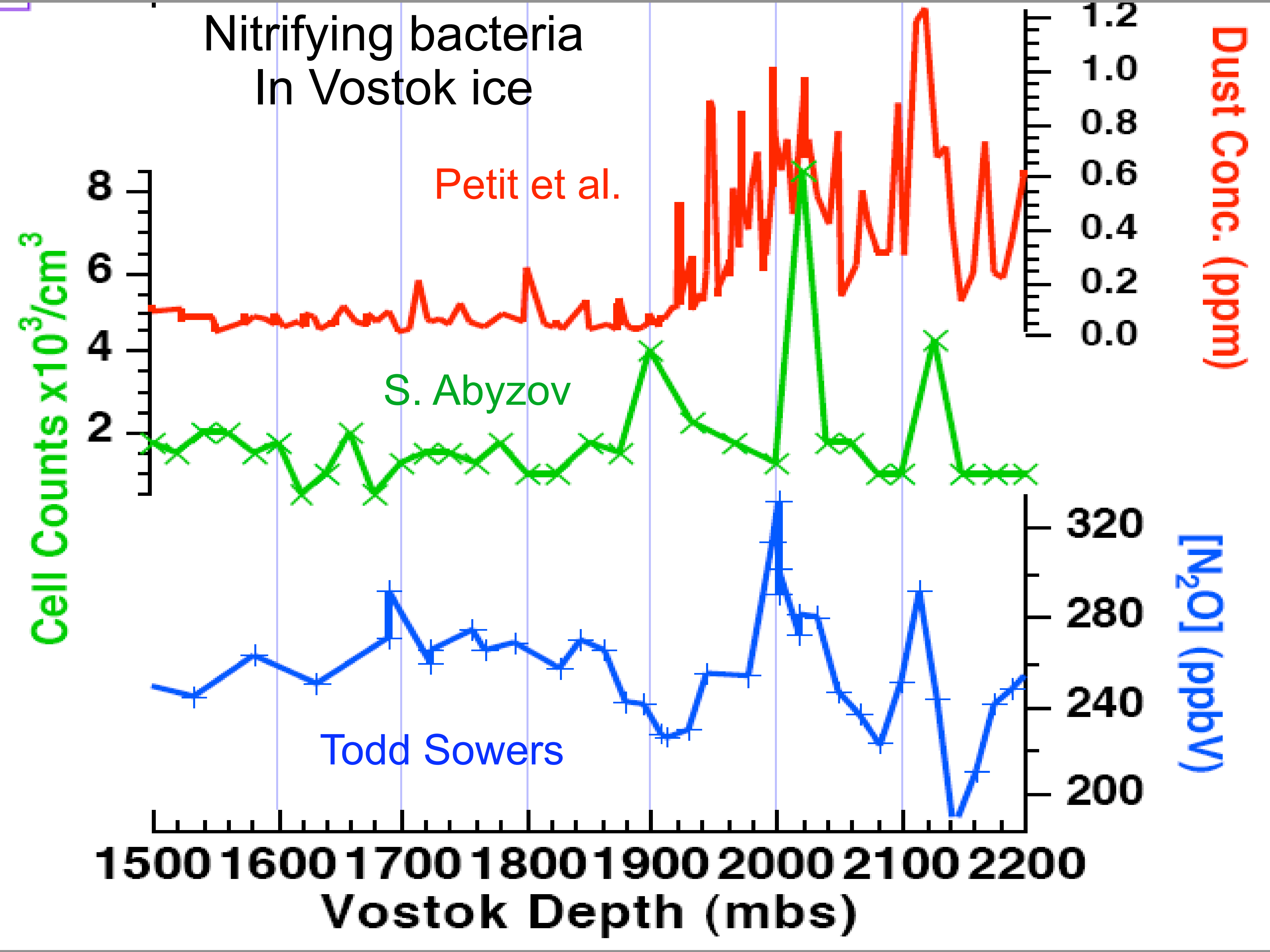
μ = metabolic rate per cell in gC gC^{-1} h^{-1}

$$\boldsymbol{\mu = \mathrm{Y_j} / n_j\, m_j\, t}$$

Y_j = metabolic yield of gas of type j;

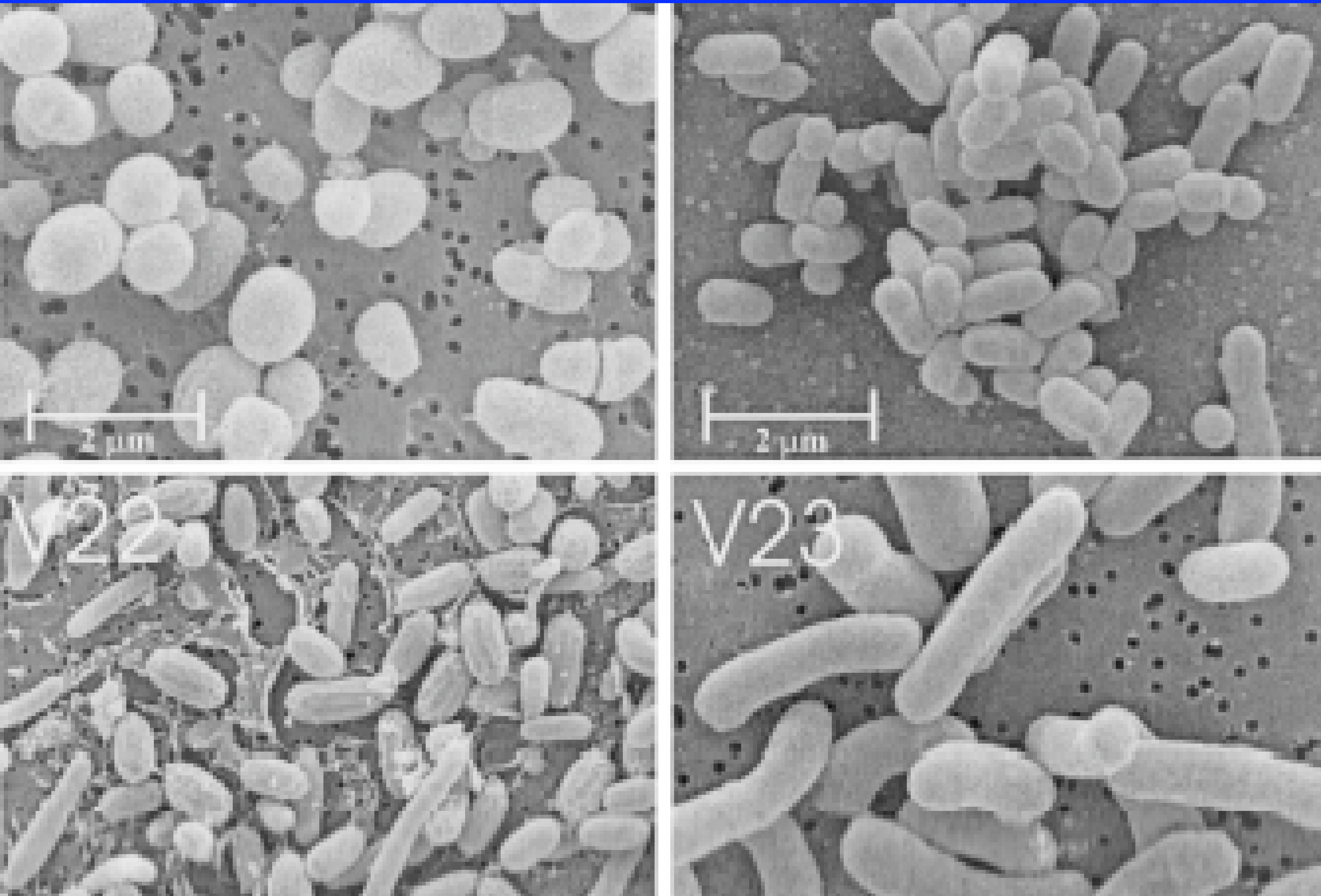
n_j, m_j = conc. and mass of microbes producing j;

t = retention time of reactants

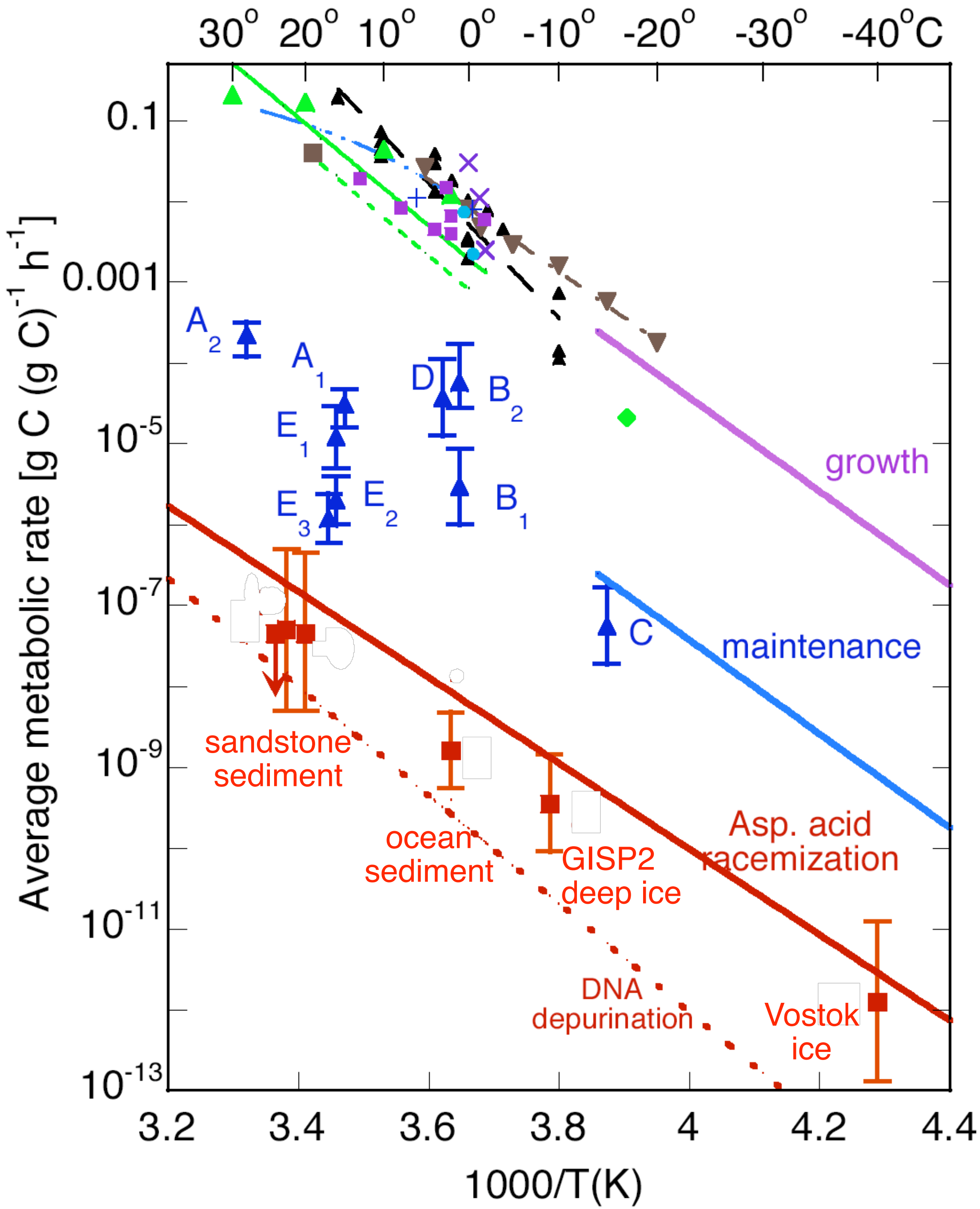


Nitrifying bacteria
In Vostok ice
Petit et al.
S. Abyzov
Todd Sowers
Cell Counts x10^3/cm^3
8
6
4
2
Dust Conc. (ppm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
[N$_2$O] (ppbV)
320
280
240
200
1500
1600
1700
1800
1900
2000
2100
2200
Vostok Depth (mbs)

SEM pictures of bacteria from Vostok ice core at 3593 m



Microbe metabolism

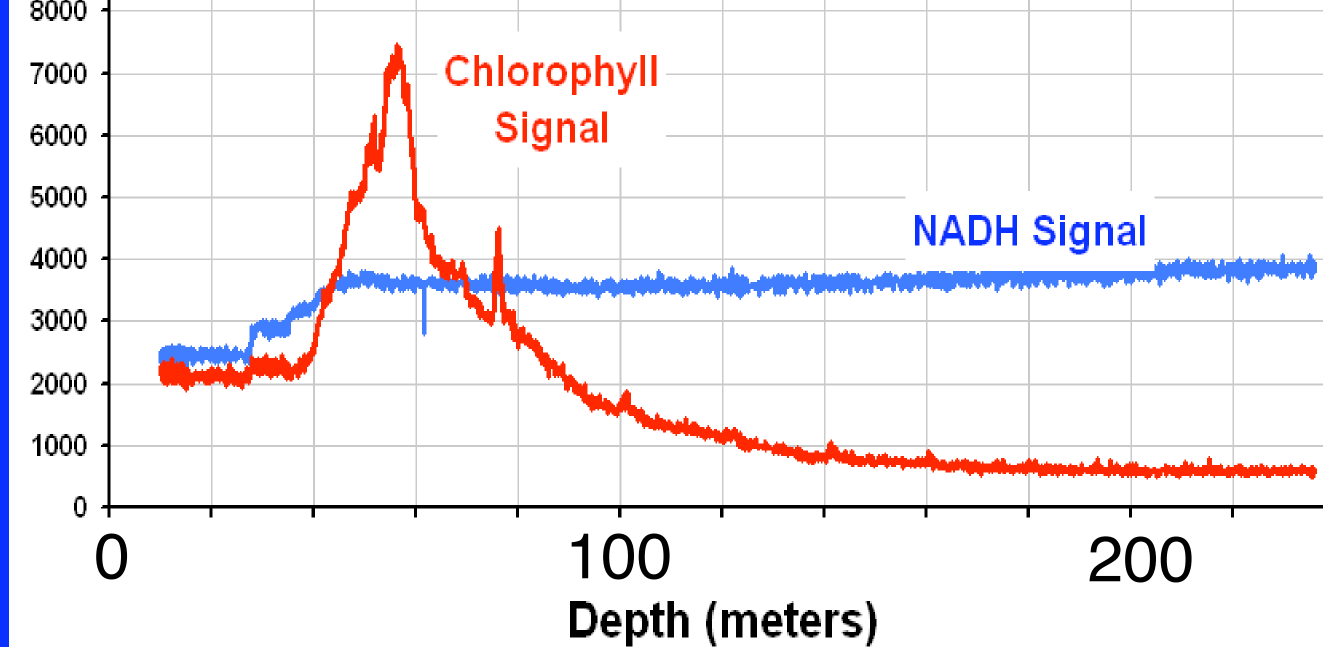


- Activation energies ~110 kJ/mole
- Rates scale as 10^6: 10^3:1
- Metabolism down to -40°C; no minimum
- In Vostok ice at -40°C, 1 turnover per 10^8 yr!
- In “survival” mode, metabolic rate for repair ≈ spontaneous damage rate.

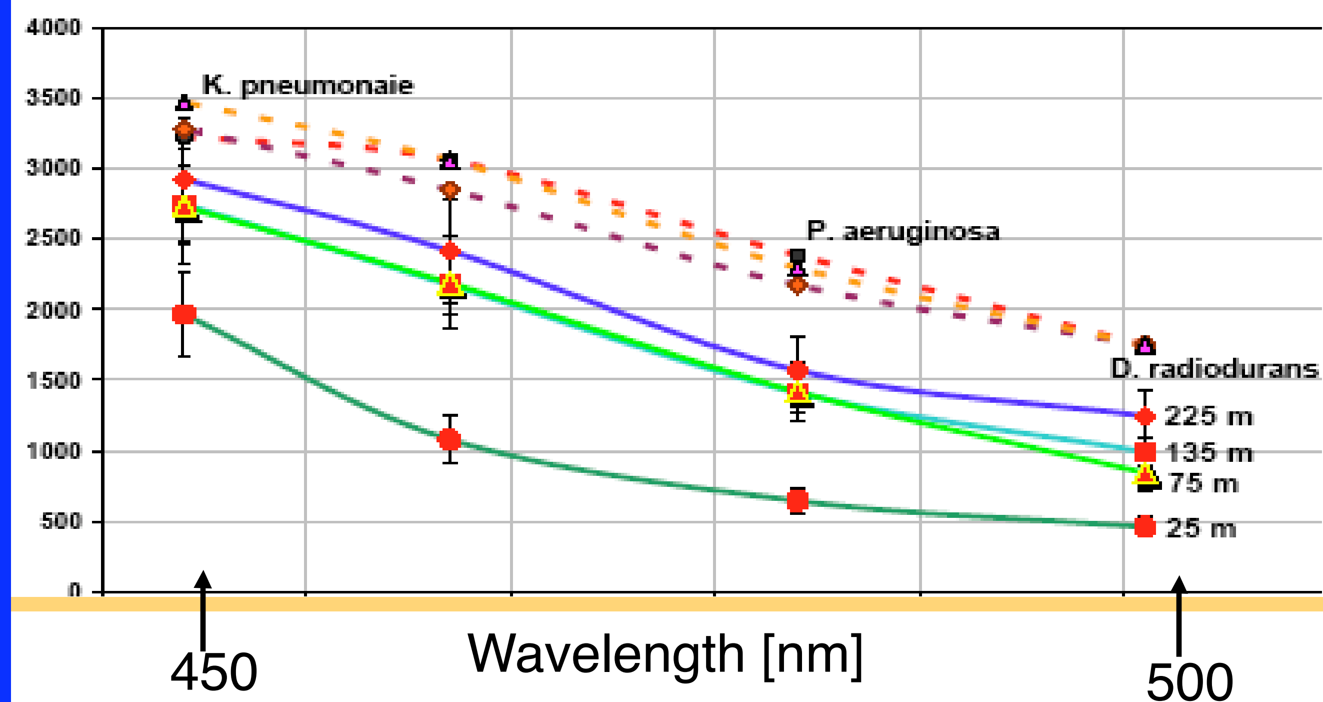
Nathan Bramall and Ryan Bay with BSL-1 at Lake Tahoe. Bramall logged a South Pole borehole with a bigger version on Jan. 16.

Log of Lake Tahoe; BSL-1 excited at 370 nm

Fluorescence from Chl-a and NADH →



NADH fluorescence spectra of known microbes compared with Tahoe microbes →



MiniBSL is a *discovery* instrument: design sensitivity ≈1 cell/cm^3.

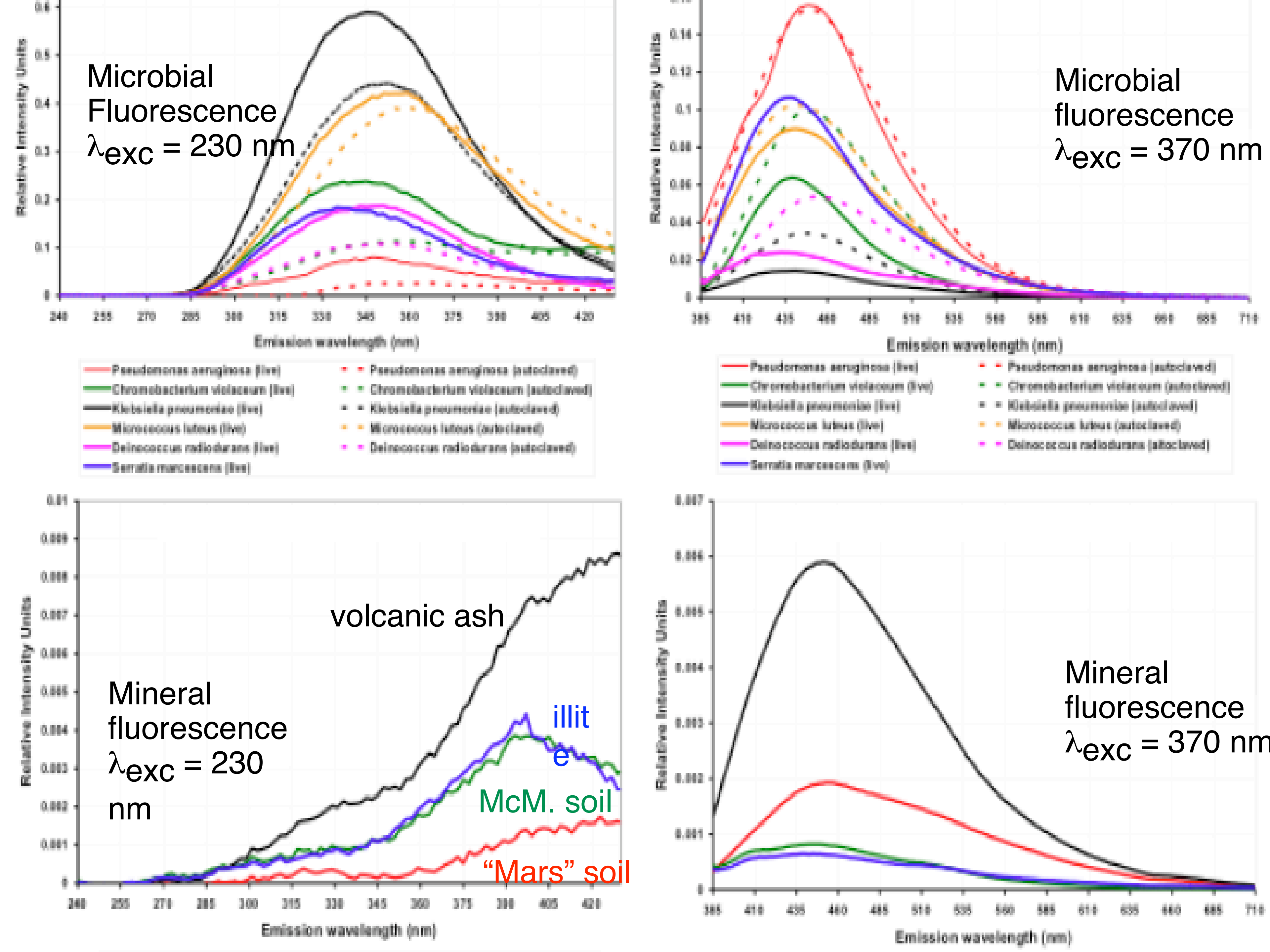
At 224 nm, microbial fluorescence dominates over mineral fluorescence. Examples:

- Chl-a ⇒ photosynthesis
- Trp ⇒ all cells
- Tyr ⇒ spore coats
- F420 ⇒ a coenzyme in methanogens and haloarchaea
- Guanosine/adenosine in acid veins ⇒ live/dead test
- PAHs

To detect other biomolecules, use other wavelengths

- Fulvic and humic acids,…
- Bacteriorhodopsin ⇒ in some haloarchaea
- NADH, flavins,…

With ND filter, scattering w/o fluorescence ⇒ dust, volcanic ash,…



Microbial Fluorescence
λexc = 230 nm
Relative Intensity Units
Emission wavelength (nm)
Pseudomonas aeruginosa (live)
Chromobacterium violaceum (live)
Klebsiella pneumoniae (live)
Micrococcus luteus (live)
Deinococcus radiodurans (live)
Serratia marcescens (live)
Pseudomonas aeruginosa (autoclaved)
Chromobacterium violaceum (autoclaved)
Klebsiella pneumoniae (autoclaved)
Micrococcus luteus (autoclaved)
Deinococcus radiodurans (autoclaved)
Microbial fluorescence
λexc = 370 nm
Relative Intensity Units
Emission wavelength (nm)
Pseudomonas aeruginosa (live)
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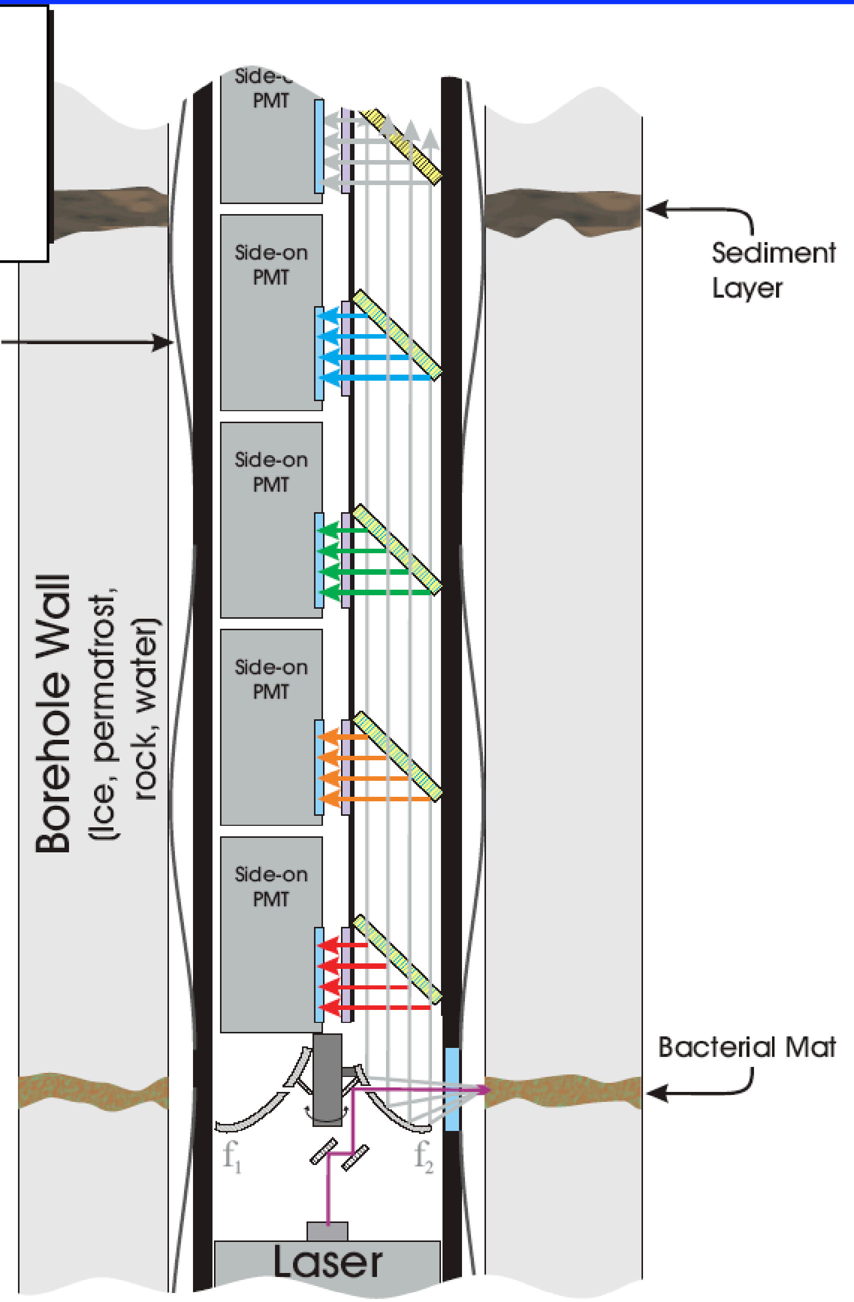
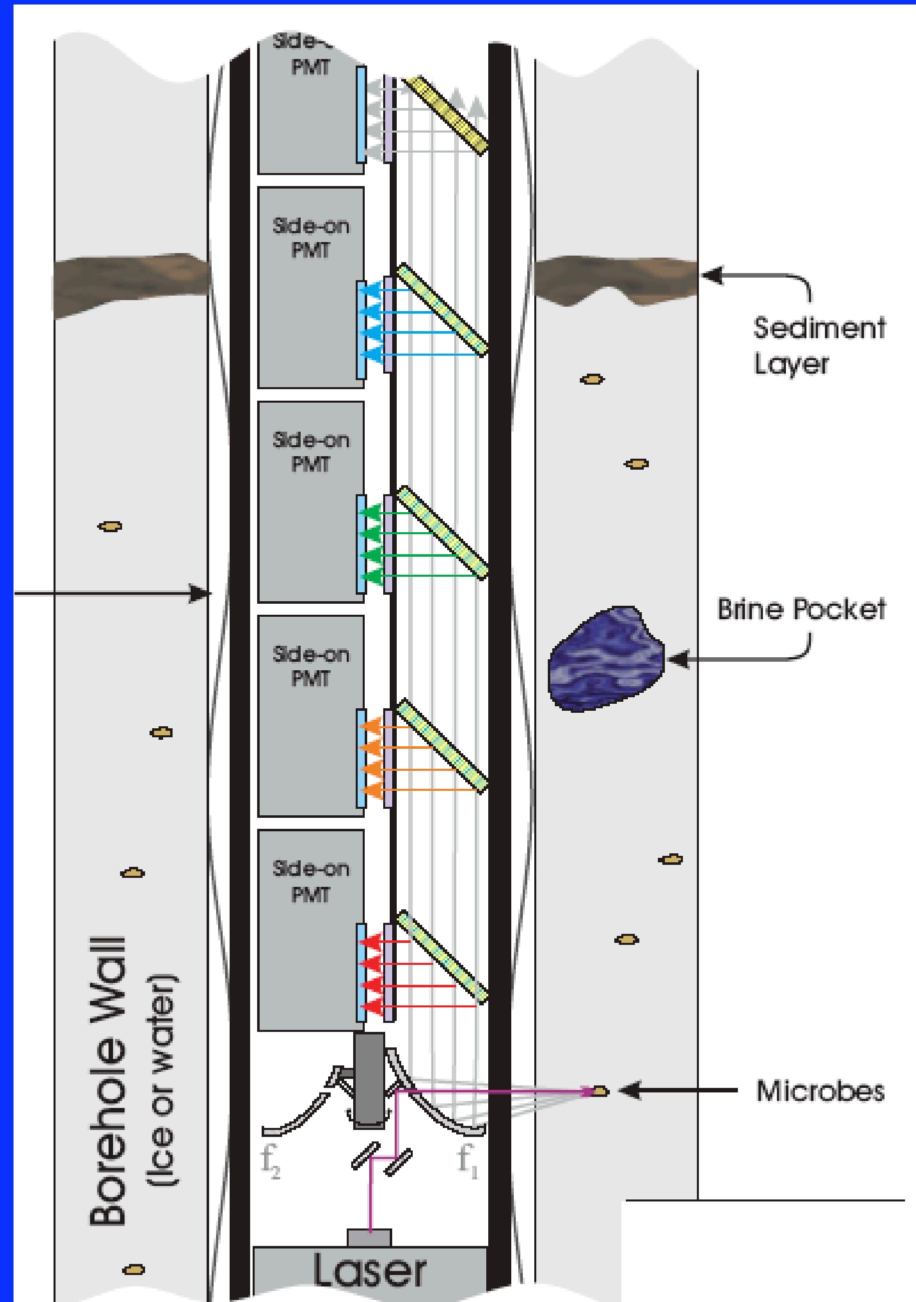
Pseudomonas aeruginosa (autoclaved)
Chromobacterium violaceum (autoclaved)
Klebsiella pneumoniae (autoclaved)
Micrococcus luteus (autoclaved)
Deinococcus radiodurans (altoclaved)
volcanic ash
Mineral fluorescence
λexc = 230
nm
illit
e
McM. soil
“Mars” soil
Relative Intensity Units
Emission wavelength (nm)
Mineral fluorescence
λexc = 370 nm
Relative Intensity Units
Emission wavelength (nm)

miniBSL

Focuses on borehole wall

Focuses inside the ice





Future potential applications

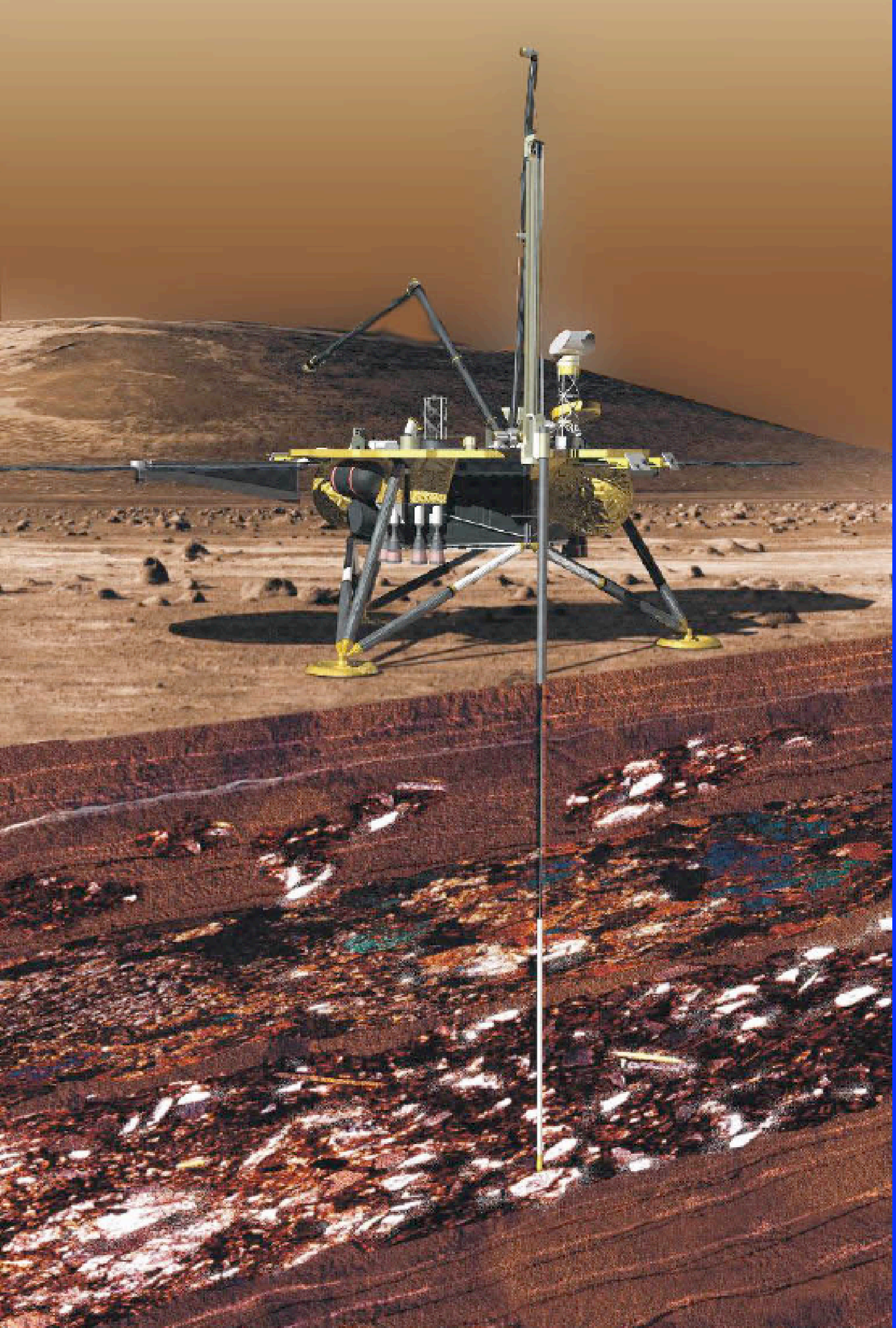
Beacon Valley (Antarctica): ~8 Myr-old permafrost/ice

Borehole in deep mine (anaerobes on walls)

Subglacial Lake Vostok (exotic microbes?)

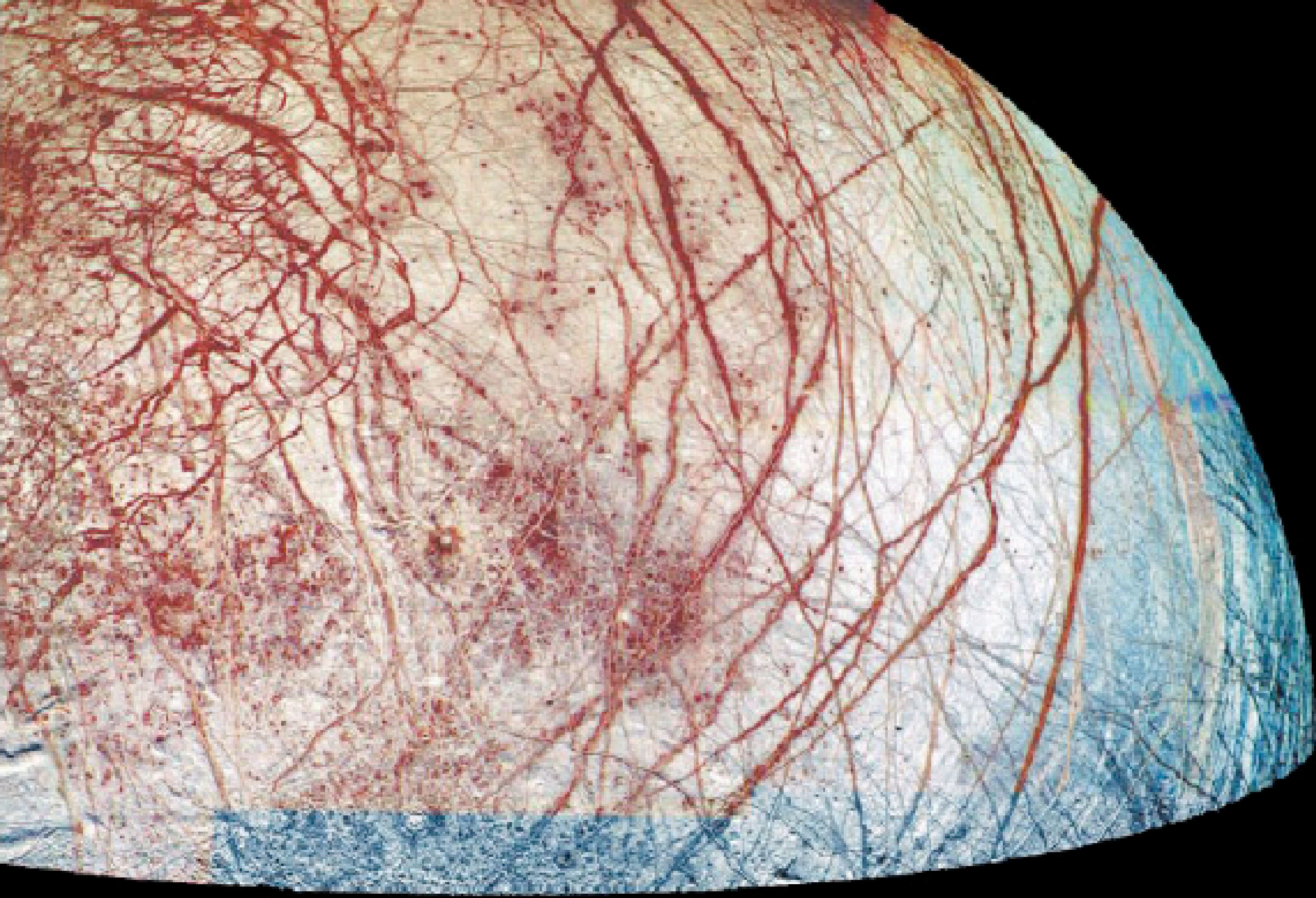
Mars subsurface?

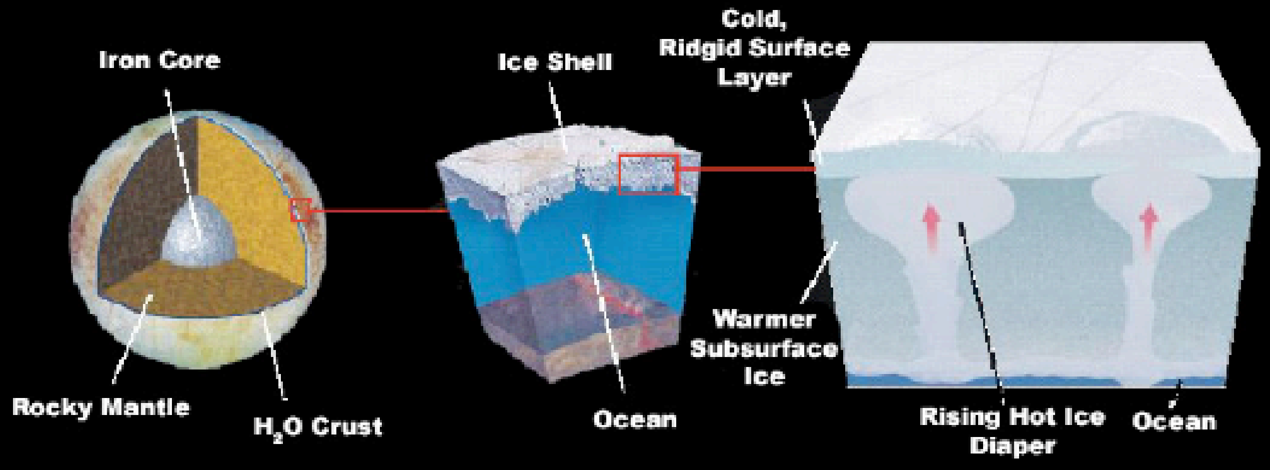
Warm diapirs in Europa’s ice?



MiniBSL could search in a Martian borehole for biomolecules in ice, permafrost, or rock and for dust and ash in clean ice.

Below the impact-gardened depth (3-14 m), organic molecules and even microbial life might avoid oxidants and surface irradiation.





Does microbial life exist in veins within warm diapirs in Europan ice?